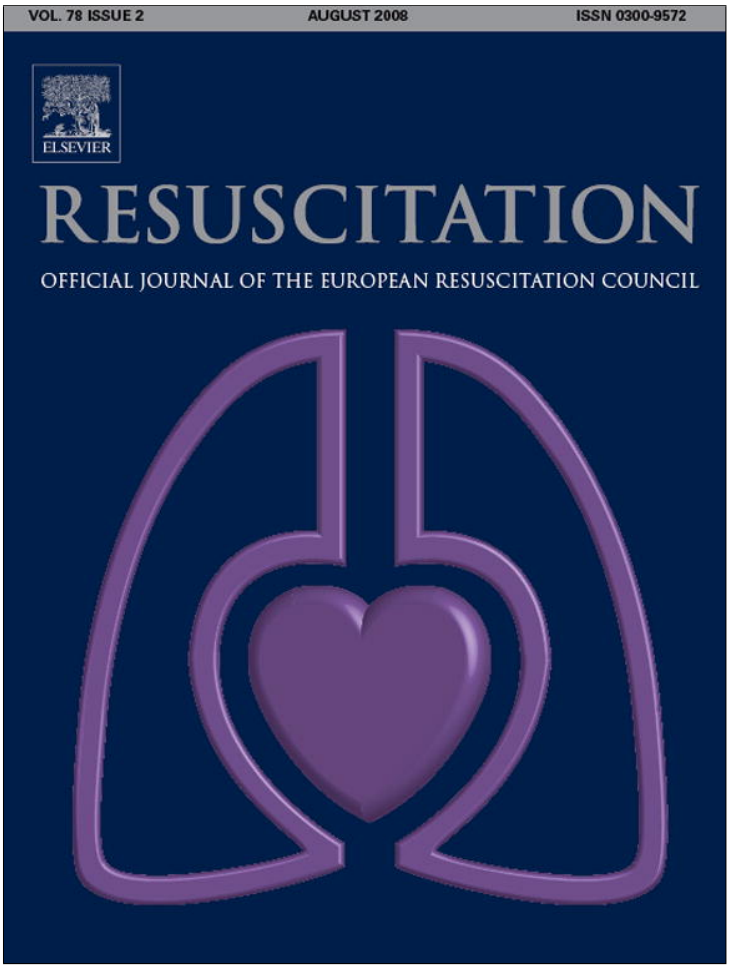

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EXPERIMENTAL PAPER

Hyperbaric oxygen improves rate of return of spontaneous circulation after prolonged normothermic porcine cardiopulmonary arrest☆

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Bretylium;
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Epinephrine (adrenaline)

Summary
Aim: This controlled, prospective, randomized porcine study tests the hypothesis that high-dose hyperbaric oxygen ($HDHBO_2$) compared with normobaric oxygen (NBO_2) or standard-dose hyperbaric oxygen ($SDHBO_2$), improves return of sustained spontaneous circulation (ROSC) after a normothermic, normobaric, 25-min, non-intervened-upon cardiopulmonary arrest. The study incorporated a direct mechanical ventricular assist device (DMVAD) for open chest continuous cardiac compressions (OCCC) to assist advanced cardiac life support (ACLS). The experiment demonstrates a dose response to oxygen concentration in the breathing mix used in resuscitative ventilation.
Materials and methods: Male pigs (average 30 kg weight) underwent a 25-min, normothermic, non-intervened-upon cardiopulmonary arrest. Following arrest all animals were ventilated with 100% oxygen and were subjected to OCCC, incorporating DMVAD-aided ACLS. The animals so treated were randomized to be in one of three groups, with six animals in each group. The NBO_2 group remained at 1.0 atmosphere absolute (ATA), while the $SDHBO_2$ and $HDHBO_2$ groups were initially placed at 1.9 and 4.0 ATA, respectively. Uniform, but not American Heart Association (AHA) protocol, ACLS was maintained as needed over the ensuing 2 h for all animals in all groups. At the end of 2 h, the animals were euthanized.

☆ A Spanish translated version of the summary of this article appears as Appendix in the final online version at doi:10.1016/j.resuscitation.2008.02.026.

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Results: Continuously sustained ROSC (mean arterial pressure $\geq$50 mmHg at all times), without the need of the pump assist over the 2-h resuscitation attempt that followed the 25-min arrest, occurred in four out of six animals in the $HDHBO_2$ group, and in none of the animals in the NBO_2 or $SBHBO_2$ groups ($p \leq 0.001$).

Conclusions: Our results show significantly sustained ROSC using $HDHBO_2$ to resuscitate swine after a 25-min, non-intervened-upon, normothermic cardiopulmonary arrest. These results could not be achieved using NBO_2 or $SDHBO_2$.

Early advances in resuscitation of patients in cardiopulmonary arrest resulted from technological improvements in airway management,[1] breathing,[2] circulation,[3] defibrillation,[4] epinephrine,[5] and hyperbaric oxygenation[6] (A, B, C, D, E, O_2). As a consequence, ''A, B, C, D, Es'' during cardiopulmonary resuscitation/advanced cardiac life support (CPR/ACLS) became codified by the American Heart Association (AHA).[7] Despite major reworking of the AHA CPR/ACLS algorithmic approach over the years, clinical outcomes today remain similar to those achieved several decades ago. Despite extensive AHA efforts for uniformity of approach, skills, and equipment in CPR/ACLS education, the M. Eisenberg prediction grid for survival after cardiopulmonary arrest from 1979 is still largely applicable today. If CPR coupled with ACLS is not initiated within 16 min of a normothermic cardiopulmonary arrest, the probability of achieving survival is zero[8] (Table 1). Thus, the estimated probability of a person who has suffered a pre-hospital cardiopulmonary arrest to be resuscitated and to leave the hospital neurologically unimpaired is 1—5%.[9–11]

Perhaps return of sustained spontaneous circulation (ROSC) after normothermic arrest is under-reported. We published a report of a 35-year-old who underwent a *normothermic* cardiopulmonary arrest about 25 min before CPR/ACLS was begun. This case involved the resuscitative use of six atmospheres of oxygen in a hyperbaric environment. A 22-year follow-up confirmed an excellent neurologic outcome.[12] Normothermic, isolated, in vitro, central nervous system (CNS) tissue[13–17] and myocardial tissue[18–21] remain viable for up to 20 min after cessation of tissue oxygen supply.

Here we tested the hypothesis that after a prolonged, normothermic, unattended cardiopulmonary arrest of 25 min in a porcine model, ventilation with oxygen in a hyperbaric environment during CPR/ACLS makes ROSC possible. Before the attempt to resuscitate normothermic cardiopulmonary arrest victims, the chance of sustained ROSC becomes less as the time of non-intervention increases. Our literature search (incorporating use of Ovid, Medline, and PubMed searching from 1950 to present) of human case reports and animal trials confirms this point. The longest controlled trial that was found for swine undergoing non-intervened-upon normothermic arrest that resulted in successful ROSC was 15 min.[22–25] Using the same search instruments and time frame, no human case reports of ROSC in a non-intervened-upon, normothermic cardiopulmonary arrest of more than 16 min could be found.[26]

Materials and methods

Animals

All 18 animals in this study were male pigs of a land-raised breed crossed with Yorkshire or Hampshire breed from a single-source herd. This study was approved by the Institutional Animal Care and Use Committees (IACUC) of the Louisiana State University Health Sciences Center in New Orleans and of the Baromedical Research Institute, New Orleans. After uniform acclimatization of the animals in a temperature-controlled, Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-approved, climate-controlled, vivarium and laboratory, food but not water was withheld overnight from the animal to be used in the experiment. On the day of the experiment, the animal was assigned by computer-generated randomization (www.randomize.net) to one of three groups: (1) 1.0 atmosphere absolute (ATA) or 100% surface equivalent fraction inspired oxygen ($SEFIO_2$) for 2 h ($n = 6$); (2) 1.9 ATA or 190% $SEFIO_2$ for 2 h ($n = 6$); or (3) 4.0 ATA or 400% $SEFIO_2$ for 15 min, then 2.8 ATA for 45 min, 1.9 ATA for 60 min ($n = 6$). The hyperbaric chamber was highly controlled and operationally arranged to be identical for all animal groups; however, it was not possible to blind members of a seasoned diving research crew to sham pressurization for the surface control group. Further, the difference in diving profiles for the pressure groups were easily distinguishable by the diving research crew. Lastly, safety considerations for defibrillation in the hyperbaric environment required procedural contingency that required the diving research crew to need to know at what depth they were.

On the day of the experiment, atropine (0.02 mg/kg) and ketamine (20 mg/kg) were injected intramuscularly. Animals were washed, dried, and transferred from the vivarium to the operating room. Continuous ear oximetry and three-lead electrocardiogram (EKG) monitoring were initiated. After a 2-s spray of the epiglottic vallecula with 20% benzocaine spray, animals were endotracheally intubated and placed on a ventilator with 95% oxygen and 5% isofluorane. The isofluorane was reduced over 10 min to a 1% concentration. A rectal thermistry probe continuously monitored temperature. Animals were placed in supine position on top of a water-circulated warming blanket to maintain rectal temperature at 37 °C. Using direct cutdown, catheters were placed in the femoral artery and the internal jugular vein for arterial manometry and for central venous/pulmonary artery manometry, oximetry and thermistry. Cardiac out-

Table 1 M. Eisenberg American Heart Association predictive chart on the probability of successful resuscitation after cardiopulmonary arrest, based on the timing of intervening cardiopulmonary resuscitation (CPR) and advanced cardiac life support (ACLS)[8]

Time to beginning of CPR after arrest (min)	Time to beginning of ACLS after arrest		
	1—8 min (%)	8—16 min (%)	>16 min (%)
1—4	43	19	10
4—8	26	19	5
8—12	—	6	0

put (CO) was measured by thermal dilution method using an Abbott Oximetrix III CO monitor (Hospira, Morgan Hill, CA). The recordings were continuously stored electronically and backed up by intermittent hand charting. The skin was incised in a vertical, mid-sternal fashion, and the thorax was opened by vertical sternotomy. The heart was released by vertical pericardotomy incision with care not to damage the vagal nerve. Since isofluorane cannot be used safely in the hyperbaric environment, the isofluorane was stopped and the animal was given intravenous (IV) chlorolose (70 mg/kg) and meperidine (5 mg/kg). Chlorolose and meperidine were chosen as an isofluorane replacement due to safety, non-volatility, and physiologic compatibility. IV xylazine was used in our pilot experiments, but this medication left the animal hemodynamically unstable in the hyperbaric environment, and accordingly was not selected for the actual experiment. The oxygen was then switched to air for continuance of mechanical ventilation.

The animal was then transferred to a 56-in. diameter, double compartment, animal/human occupancy hyperbaric chamber. All of the continuous monitoring modalities were maintained by means of through-hull fittings for connection to topside (exterior) electronic recording devices. Each animal was accompanied in the hyperbaric chamber by human attendants at all times throughout the experiment. The animals were fibrillated by a catheter wire placed directly on the right ventricular epicardium, through which a 70-V alternating current (ac) was administered.

The ventilator was then stopped and disconnected from the animal's endotracheal tube. A normal saline solution, IV drip at 75 cm^3/h, was infused by central venous catheter. Twenty-five minutes were allowed to elapse without any other intervention. All animals uniformly deteriorated from induction of ventricular fibrillation to the development of asystole over the 25-min period. All animals had complete cessation of ventilation over the 25-min period. After each animal sustained a 25-min period of inattention, each pulseless and apneic animal was placed back on the ventilator with 100% oxygen. At the same time, a direct mechanical ventricular assist device (DMVAD)[27] (modified for this experiment to work in the hyperbaric as well as the normobaric environment) was attached (Figure 1). This device was used for several reasons. First, when initially applied, it produced consistently similar CO in asystolic swine hearts. Second, the DMVAD mechanically standardized the cardiac compressions given to all animals, averting the variability inherent in hand-generated cardiac compressions. Third, since the open chest method of CPR was required for use of the DMVAD, it could be quickly disengaged for direct observation of heart wall motion should it occur. Fourth, the DMVAD used was not a high performance device, but rather approximated the production of compressions that would be expected from effective continuous closed chest cardiac compressions (CCC-CPR).[28]

In every instance on every animal, the DMVAD was cycled at 80 compressions per minute of equal strength and duration. At 30 min post-ventricular fibrillation induction, in-series, one-time boluses of IV bretylium tosylate (5 mg/kg), IV epinephrine (0.02 mg/kg), and IV lidocaine (2 mg/kg) were given. The antiarrhythmic agent, bretylium tosylate, was selected for this experimental model for its observed effectiveness in ablating dysrrhythmias in cases of mild hypothermia.[29] Each animal uniformly remained in a 23.0 °C environment during the prolonged 25-min cardiopulmonary arrest, and each animal's core temperature (rectal) consistently dropped 1 °C during this period. The experiment was conducted between 1998 and 2005 when bretylium tosylate was available for animal use. The bretylium tosylate was initially obtained from Astra in Westborough, MA, 01581, and after 2000 was available from Sigma—Aldrich in St. Louis, MI, 67178, until 2005 when it was discontinued. Lidocaine was used to lessen the deleterious effect of arterial gas embolism, should it occur as caused by accidental

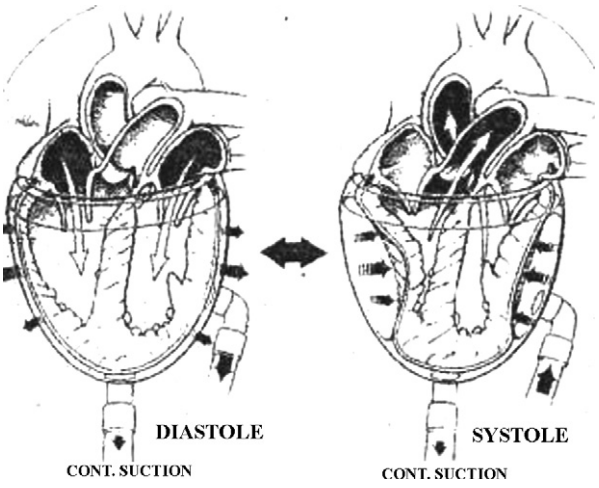

Figure 1 Direct ventricular assist device. In swine, this device is capable of producing cardiac output of 20—30% of pre-arrest levels in a fibrillating or asystolic heart. (Reprinted with permission from Lippincott, Williams & Wilkins. Anstadt MP. Direct mechanical ventricular assistance promotes salvage of ischemic myocardium Trans Am Soc Artif Intern Organs 1987;33:721.)

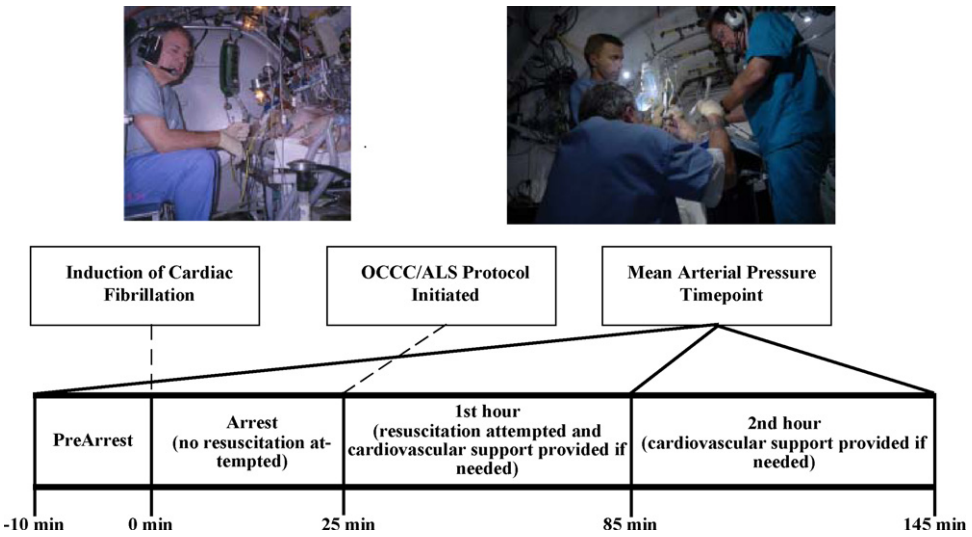

Figure 2 Timeline of experimental design relative to initiation of cardiac fibrillation. Data were recorded when the animal was stable in the pre-arrest condition for 10 min; then the heart was arrested and mechanical breathing stopped for 25 min; then the ACLS/HBO_2 therapy resuscitation protocol was performed for 2 h; finally, the animal was euthanized.

pulmonary overpressurization by ventilation of these open chest animals in the hyperbaric environment.[30]

At 30-min post ventricular fibrillation, if needed, the animal was defibrillated by directly applied cardiac paddles placed upon epicardial surface with up to three successive 20-, 35-, and 50-joule shocks. Thereafter, at intervals of 3 min for a total of two more doses, if needed, follow-up IV epinephrine (0.02 mg/kg) was given. At 40 post-arrest minutes the animal, if needed, was defibrillated with up to three more successive 50-joule shocks. At 45 min, if needed to improve mean arterial pressure (MAP), an epinephrine drip (0.1 μg/(kg min)) was begun. Animals with the DMVAD in place were periodically checked to determine whether ventricular fibrillation, asystole, or pulseless electrical activity (PEA) persisted. For purposes of this study, we defined PEA as a regular, cardiographic rhythm that did not produce a MAP $\geq$50 mmHg. If these conditions persisted, the DMVAD was continued, and if not present, the DMVAD was removed, and achievement of sustained ROSC was declared if the MAP continuously remained $\geq$50 mmHg. The total post-arrest CPR/ACLS resuscitation period (beyond the 25-min period of unattended cardiopulmonary arrest) was 2 h, at which point the animal's heart was asystolic or fibrillating or was electrically fibrillated to allow euthanasia. The brain was removed by 1-min access by a large, powered, $2\frac{3}{4}$-in. diameter trephinator to allow for the possibility of future laboratory evaluation of brain tissue. Each animal, either on its own by sustained ROSC (or if without ROSC then by DMVAD), maintained measurable MAPs for the entire 2-h, post-25-min arrest period. The timeline for the experimental design is shown in Figure 2.

The diving profiles in the hyperbaric chamber during the 2-h resuscitative effort were by pressure exposure alike for human attendants and animals. The only difference was that the animals were ventilated with pure oxygen, while the humans breathed air using oxygen at proscribed times to assist decompression safely. Table 2 provides a verbal walk-through comparatively of the dive exposures for both the animals and their human attendants for each treatment group.

It is unfortunate that full evaluation of the brain tissue was impossible. Hurricane Katrina flooded the building containing the −80 °C freezers where the tissues were stored, and the samples were destroyed after 2 months of having no electrical supply to the freezers. Before the storm, analysis of every animal's brain tissue was completed for levels of thiobarbituric acid reactive substance (TBARS), myeloperoxidase (MPO), and poly ADP-ribose polymerase (PARP).

Equal weights of frozen brain samples from each animal were assayed for levels of malonaldehyde by method of W. Wascowicz[31] using the Oxi-tek TBARS assay kit made by the Zeptometrix Corporation of Buffalo, NY, 14202. Equal weights of frozen brain samples from each animal were assayed for levels of myeloperoxidase by method of K. Chatzipanteli[32] using the antibody kit from DAKO Carpinteria of California, 93013. To perform the Western-Blot Analysis for PARP degradation, frozen brain samples were assayed to determine the extent of cleavage of PARP by measuring density of PARP, a caspase-3 substrate[33,34] using a PARP antibody kit (Santa Cruz Biotech, Los Angeles, CA, 95060). Results of these analyses are reported in Figures 3—5.

Statistical analysis

MAP was analyzed with a two-factor analysis of variance (ANOVA) with a nested arrangement of treatments.[35] The main effects were treatment (normobaric oxygen (NBO_2), standard-dose hyperbaric oxygen ($SDHBO_2$), high-dose hyperbaric oxygen ($HDHBO_2$)) and time (three periods of observation during the course of the experiment); the interaction of treatment and time was also included in the ANOVA model. The blocking effect of subjects was ''subjects within treatment'' (pigs within pressure level), which was the nested arrangement of treatments. All stated *p*-values were from F-tests comparing variances of the

Table 2 Comparative animal and human attendant pressure and oxygen exposures for each treatment group

NBO_2 group animals	After the 25-min animal arrest by induction of ventricular fibrillation, the animals stayed at 1 ATA for the subsequent 2-h resuscitative effort. During the 2-h period, the animals were ventilated with pure oxygen. Immediately at the beginning of the 2-h period, the DMVAD was turned on and IV drugs were pushed. Five minutes later, the first defibrillatory attempt was made.
NBO_2 group human attendants	After the 25-min arrest of the animal, the human attendant stayed in the chamber breathing only air at 1 ATA for the entire subsequent 2-h resuscitative effort.
$SDHBO_2$ group animals	After 20 min into the cardiopulmonary arrest induced by ventricular fibrillation, the apneic animal was pressurized over 5 min to 1.9 ATA. Reaching 1.9 ATA and 25 min into the arrest, the ventilator was turned on to begin ventilation with pure oxygen. Likewise, the DMVAD was turned on at this point and IV drugs were pushed. Five minutes later, the first defibrillation attempt occurred. The animal remained on the ventilator on pure oxygen for the duration of the dive.
$SDHBO_2$ group human attendants	After 20 min into the arrest of the animal, the human attendant on air accompanied the apneic animal by pressurization over 5 min to 1.9 ATA. At 1.9 ATA, by then 25 min after induction of ventricular fibrillation in the animal, the human attendant continued breathing compressed air for 80 min and then went on oxygen for 15 min and then air for 5 min. At this point, the human attendant went on oxygen again and ascended with the animal over 15 min to the surface.
$HDHBO_2$ group animals	20 min into the cardiopulmonary arrest induced by ventricular fibrillation, the animal was pressurized over 5 min to 4.0 ATA. Only after reaching 4.0 ATA at 25 min into the arrest was the ventilator turned on to begin ventilation with pure oxygen. Likewise, the DMVAD at this point was turned on and the IV drugs were pushed. Five minutes later the first defibrillation attempt occurred. The animal remained on pure oxygen ventilation for the entire remaining dive period to include a total 10-min exposure at 4.0 ATA, followed by a 2-min ascent on oxygen to 2.8 ATA, then on oxygen for 43 min, followed by a 10-min ascent on oxygen to 1.9 ATA, then again oxygen for 35 min, followed by a 15-min ascent on oxygen to surface.
$HDHBO_2$ human attendants:	After 20 min into the cardiopulmonary arrest of the animal, the human attendant while breathing air, accompanied the apneic animal in pressurization to 4.0 ATA, which was by then 25 min after induction of arrest of the animal. The human attendant remained on air for a 10-min exposure at 4.0 ATA. The human attendant then ascended over 2 min on air to 2.8 ATA and remained there on air for 43 min and then on air ascended to 1.9 ATA. At 1.9 ATA on oxygen the human attendant remained 20 min and then went on air 5 min and then back on oxygen for a 15 min ascent to surface.

blocking effect, main effects, and interactions. The main effect of treatment was F-tested using the variability due to pigs within treatment as a denominator and all other effects were F-tested using the overall error variance as the denominator. Post hoc tests of main effect and interaction mean levels were conducted using protected *t* tests and a simulation method of adjusting alpha levels for multiple comparisons.[36]

Results

Sustained ROSC was defined for purpose of this study as the ability of the unassisted heart to continuously maintain a MAP of $\geq$50 mmHg in concert with a normalized EKG and observable strong heart wall motion. There were significant differences among the three groups of animals for continuously sustained ROSC. The $HDHBO_2$ group compared to either the NBO_2 or $SDHBO_2$ group had a significant increase in the rate of ROSC ($p \leq 0.001$). There was no significant difference in occurrence of either initial or sustained ROSC between the NBO_2 and $SDHBO_2$ groups. All animals in the NBO_2 and $SDHBO_2$ groups required the DMVAD to be kept in place to support the failed circulation during the entire 2-h resuscitation attempt. Only two animals in the $HDHBO_2$ group required this intervention, one of which only needed the pump briefly, toward the end of the 2-h resuscitative attempt. The other four $HDHBO_2$ animals required the DMVAD for only a brief period at the onset of resuscitation, after which it could be completely and permanently

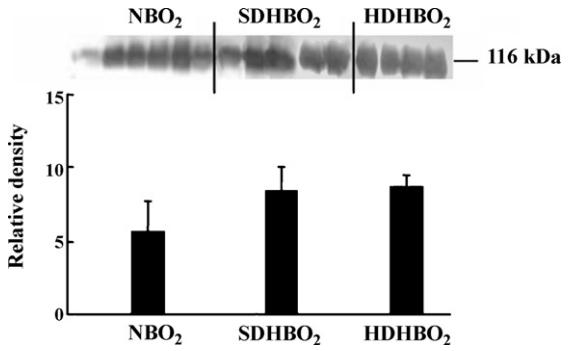

Figure 3 Cleavage of poly ADP-ribose polymerase, the caspase-3 substrate, in pig brain extracts. The average relative density of PARP in the NBO_2, $SDHBO_2$, and $HDHBO_2$ groups were 5.71, 8.39, and 8.63, respectively. The NBO_2 group had a statistically lower relative density of PARP than did the $SDHBO_2$ or $HDHBO_2$ group ($p = 0.04$), indicating higher caspase-3 activity and greater apoptosis. Both oxygen groups shows lowest caspase activity and greater PARP than respective findings in control groups.

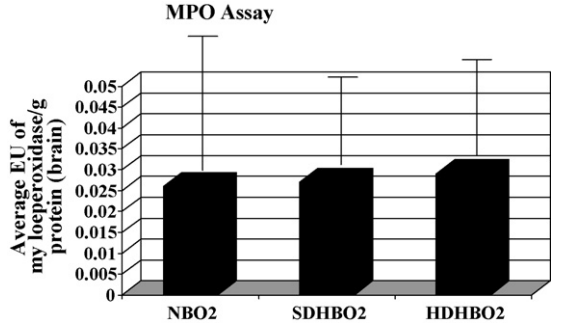

Figure 4 Myeloperoxidase (MPO) assay. The levels of myeloperoxidase represent quantitatively the number of leukocytes entrapped in the CNS microvasculature after reperfusion after the prolonged cardiopulmonary arrest. Myeloperoxide values represented by EU/g of extracted protein from the animal's brain tissue is recorded on the *y*-axis. Statistical evaluation using one-way analysis of variance evidenced no statistically significant difference among groups ($p > 0.05$).

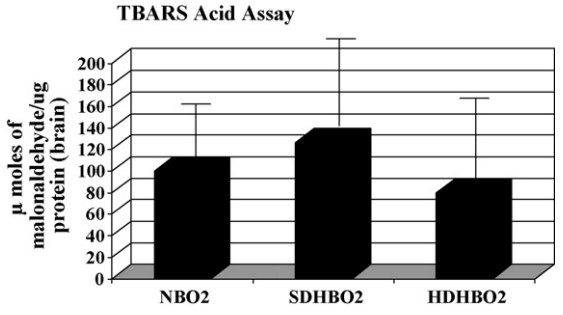

Figure 5 TBARS assay. Lipid peroxidation was quantitated by the previously referenced TBARS acid assay. Malonaldehyde was measured in μmol/μg extracted protein of brain tissue in each animal. Values are represented on the *y*-axis. Results were significant by using a two-tailed student *T*-test comparing the results of the $HDHBO_2$ group to the $SDHBO_2$ and NBO_2 groups ($p \leq 0.043$).

removed. Post-arrest CO in the DMVAD-unassisted hearts was only achievable in the $HDHBO_2$ group animals; the results are depicted in Figure 6.

CO was measured before, during and after the arrest period. After resuscitation was begun, at all times, all the animals in the NBO_2 and $SDHBO_2$ groups needed the DMVAD to be fully engaged and operational for MAP or CO of any extent to be achieved. In the two groups without any sustained ROSC, MAP of zero and CO of zero were achieved without the DMVAD engaged. Even in the animals in the NBO_2 and $SDHBO_2$ groups, there was a difference in efficacy of the DMVAD to generate COs in the uniformly asystolic hearts of both groups. Perhaps relative states of hypoxia made a difference in heart wall compliance among the groups. In the $HDHBO_2$ group, four out of six animals had a sustained ROSC with MAP >50 mmHg. Five out of six animals had continuous measurable CO throughout the 2-h resuscitative effort past the 25-min, unintervened-upon cardiopulmonary arrest period. Results are shown in Figure 7.

Other factors were measured throughout the experiment, to include arterial oxygen partial pressure (PaO_2) and pH. Please see Tables 3 and 4 for these results.

Discussion

Hyperbaric oxygen (HBO_2) for cardiac resuscitation was first used in a prehospital, mobile cardiac unit before the advent of lidocaine and thrombolytics. In these instances, HBO_2 was observed to rapidly ablate reperfusion dysrhythmias.[6] Recent controlled canine thrombolytic studies duplicated the HBO_2-induced ablation of reperfusion dysrhythmias and also salvaged heart wall.[37] Further, HBO_2 was effectively used in England to resuscitate moribund, apneic neonates, leaving them without apparent sign of brain injury.[38] The clinical demand for this technology quickly exhausted the very few clinicians who were trained in monoplace (single person chamber) critical-care patient management. The threat of accident or injury from the high-pressure oxygen was also an impediment. Despite dramatic human success by the use of HBO_2 in the above instances, caution prevailed and 1.0 ATA oxygen became the standard of care as an AHA class I ACLS drug.

Ironically, 1.0 ATA oxygen was approved in the AHA CPR/ACLS guidelines many years ago, and then became an AHA-endorsed, evidence-based, class I ACLS drug. Very appropriately the question arose: ''Would 1.01 ATA oxygen ($SEFIO_2$ 101%) be a better or at least equally effective dose.'' This study does not answer that question, but instead explores whether 1.9 ATA oxygen or 4.0 ATA oxygen administered in a multiplace hyperbaric chamber (to allow hands-on care of the arrest subject) confers any advantage over 1.0 ATA 100% oxygen. Subsequently, HBO_2 has been used in controlled animal studies pre-arrest[39] and post-resuscitation[40] to avert myocardial and brain damage, respectively. Both models produced markedly decreased CNS reperfusion injury compared to that found in normobaric, oxygen-treated controls. No studies have examined HBO_2 for an acute resuscitation treatment until a 1988 controlled experiment using a 15-min, non-intervened-upon cardiopulmonary arrest in guinea pigs.[41] This study achieved results similar to our present study and demonstrated that HBO_2

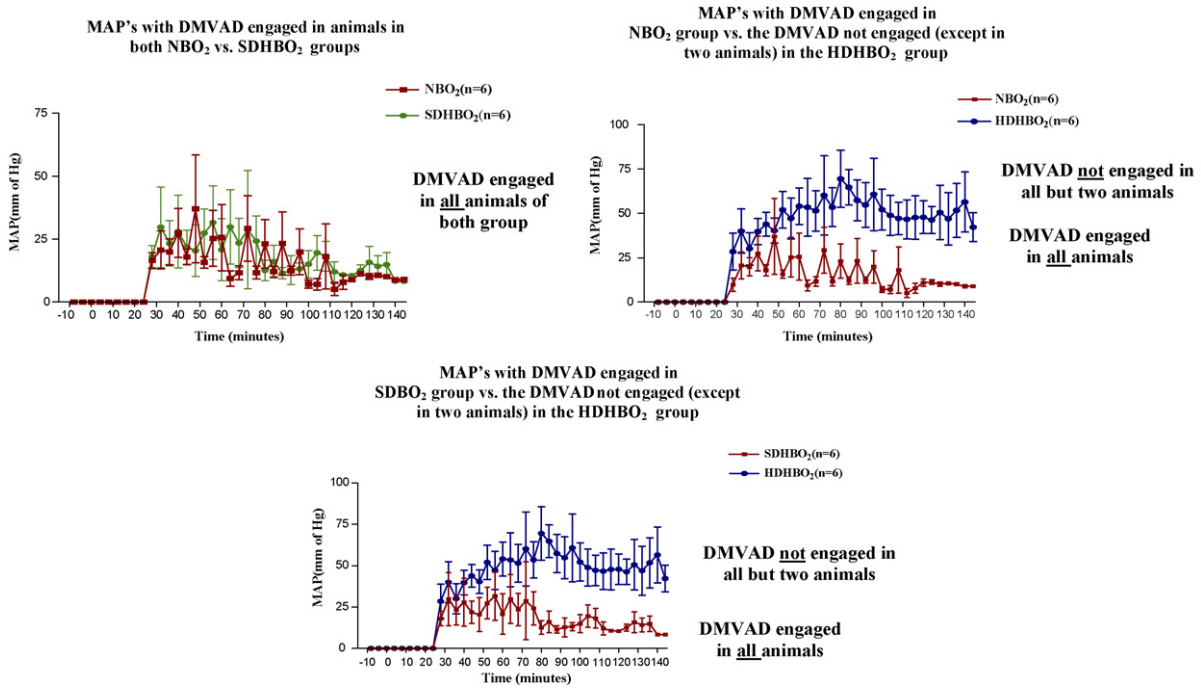

Figure 6 Average MAP over time for the animals in NBO_2, $SDHBO_2$, and $HDHBO_2$ group. MAPs were monitored throughout the experiment. All groups remained in cardiac arrest for 25 min with zero MAP, after which, ACLS protocol was initiated for all groups and continued as indicated. Both NBO_2 and $SDHBO_2$ animals required ACLS and external mechanical cardiac pumping throughout the duration of the experiment (120 min of the resuscitative effort). During this resuscitative effort, MAPs for NBO_2 and $SDHBO_2$ animals were not different (A); however, for the $HDHBO_2$ group MAPs were statistically significant in difference from the NBO_2 and $SDHBO_2$ groups ($p < 0.001$).

doses higher than 1.9 ATA oxygen were required for ROSC and immediate survival.

Fire/explosion and pulmonary oxygen toxicity are safety concerns in a hyperbaric chamber. The chamber must have a powerful water-deluge fire-suppression system and built-in breathing system to protect occupants from toxic fumes resulting from fire or explosion. During the course of this and past experiments, our team administered well over 1240 defibrillatory shocks in 4.0—6.0 ATA compressed air while animals were mechanically, endotracheally ventilated with 100% oxygen; no fire or combustion occurred. Only infrequent, minimal arcing was detected between the paddle and epicardium. We feel this safety record was facilitated by the use of lower voltages in the open chest model as compared to closed chest. Oxygen toxicity can be markedly diminished by intermittency of HBO_2. Intermittency has markedly decreased the incidence of seizure and pulmonary oxygen toxicity.[42] All human drug profiles for attendants accompanying the animals were analyzed with the Nobendem computerized table analysis for assurance of safe decom-

Table 3 Average of measured arterial PaO_2 and $PaCO_2$ determinations by group for pre-arrest and 1 h, post-induction of ventricular fibrillation periods

	Average baseline arterial $PaO_2/PaCO_2$ pre-arrest	Average baseline arterial $PaO_2/PaCO_2$ 1 h post-induction of ventricular fibrillation
NBO_2 group	400 mmHg/36.9 mmHg	219 mmHg/45.4 mmHg
$SDHBO_2$ group	381 mmHg/42.5 mmHg	504 mmHg/50.5 mmHg
$HDHBO_2$ group	394 mmHg/40.6 mmHg	1313 mmHg/40.6 mmHg

Table 4 Average of arterial pHs determinations by group for pre-arrest and 1 h, post-induction of ventricular fibrillation periods

	Average baseline arterial pH pre-arrest	Average arterial pH 1 h post-induction of ventricular fibrillation
NBO_2 group	7.46	6.99
$SDHBO_2$ group	7.38	7.07
$HDHBO_2$ group	7.44	7.10

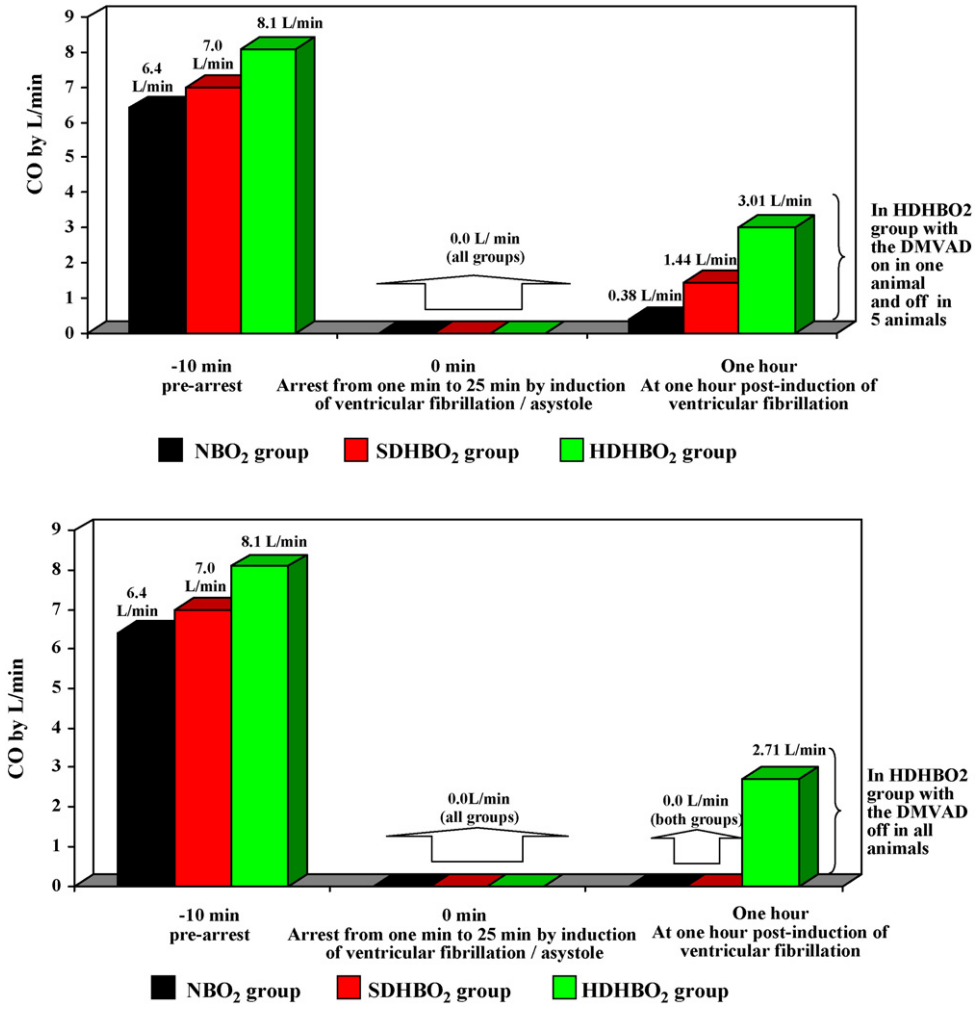

Figure 7 Average group cardiac output. For each group of animals in this experiment, baseline CO measurements were obtained just before subjecting the animals by randomization to ACLS effort by ventilation with either NBO_2, $SDHBO_2$, or $HDHBO_2$ in a hyperbaric chamber. At 1 h after the start of the 25-min, non-intervened-upon cardiopulmonary arrest (or at 35 min after completion of a 25-min, non-intervened-upon cardiopulmonary arrest) CO measurements were performed on each animal in the NBO_2 and $SDHBO_2$ groups, with and without the DMVAD engaged. At this exact same time, in the $HDHBO_2$ group, the hearts were all beating and producing continuously sustained blood pressure except for one animal in PEA; for this reason, measurements of CO were then made without the DMVAD engaged in five animals with beating hearts productive of MAP $\geq$50 mmHg. In the animal in the $HDHBO_2$ group with PEA, CO measurement was made with and without the DMVAD engaged as in all the animals in the NBO_2 and $SDHBO_2$ groups. In the $HDHBO_2$ group, at 1 h post-induction of ventricular fibrillation without the DMVAD engaged, all animals but one outperformed any animal in the NBO_2 and $SDHBO_2$ groups. Post-induction of ventricular fibrillation, the animals in the $HDHBO_2$ group with the pump off had an average CO of 2.31 L/min (CI of 5.21 L/min). If the pump were engaged on the one animal with PEA in this group, the animals' group average CO would have been 3.01 L/min (CI of 5.79 L/min). Without the DMVAD engaged in the one animal with PEA, the average 1-h, post-induction of ventricular fibrillation CO of the $HDHBO_2$ group dropped to 2.71 L/min (CI of 5.21 L/min), compared to the 0.0 L/min of both the NBO_2 and $SDHBO_2$ groups without the DMVAD engaged. Lastly with the DMVAD engaged on just one persisting PEA animal in the $HDHBO_2$ group, the 1-h, post-induction of ventricular fibrillation CO averaged 3.01 L/min (CI of 5.79 L/min) compared to the DMVAD engaged average of 1.44 L/min (CI of 2.77 L/min) in the $SDHBO_2$ group and 0.38 L/min (CI of 0.73 L/min) in the NBO_2 group. The *T*-test for equality of means by independent samples test yielded $p \leq 0.007$ for these results.

pression (http://scuba-doc.com/NOBENDEM.pdf). The dive profile of the human attendants is presented in the dive profile diagram in Figure 8. Humans can easily tolerate a unit pulmonary oxygen toxicity dose (UPTD) during a single hyperbaric exposure of 300 UPTDs without sustaining detectable lung injury. An UPTD is defined as 1-min of breathing 100% oxygen at one atmosphere equivalent for 1 min.[43] Neither the human attendants nor the swine exceeded a UPTD of over 300 during the hyperbaric exposures used in this trial (see Table 5).

HBO_2 has beneficial effects on acute ischemic pathophysiology. HBO_2 has been shown to inhibit reperfusion injury through its action on polymorphonuclear neutrophils.[44] In a carbon monoxide model this was due to a transiently

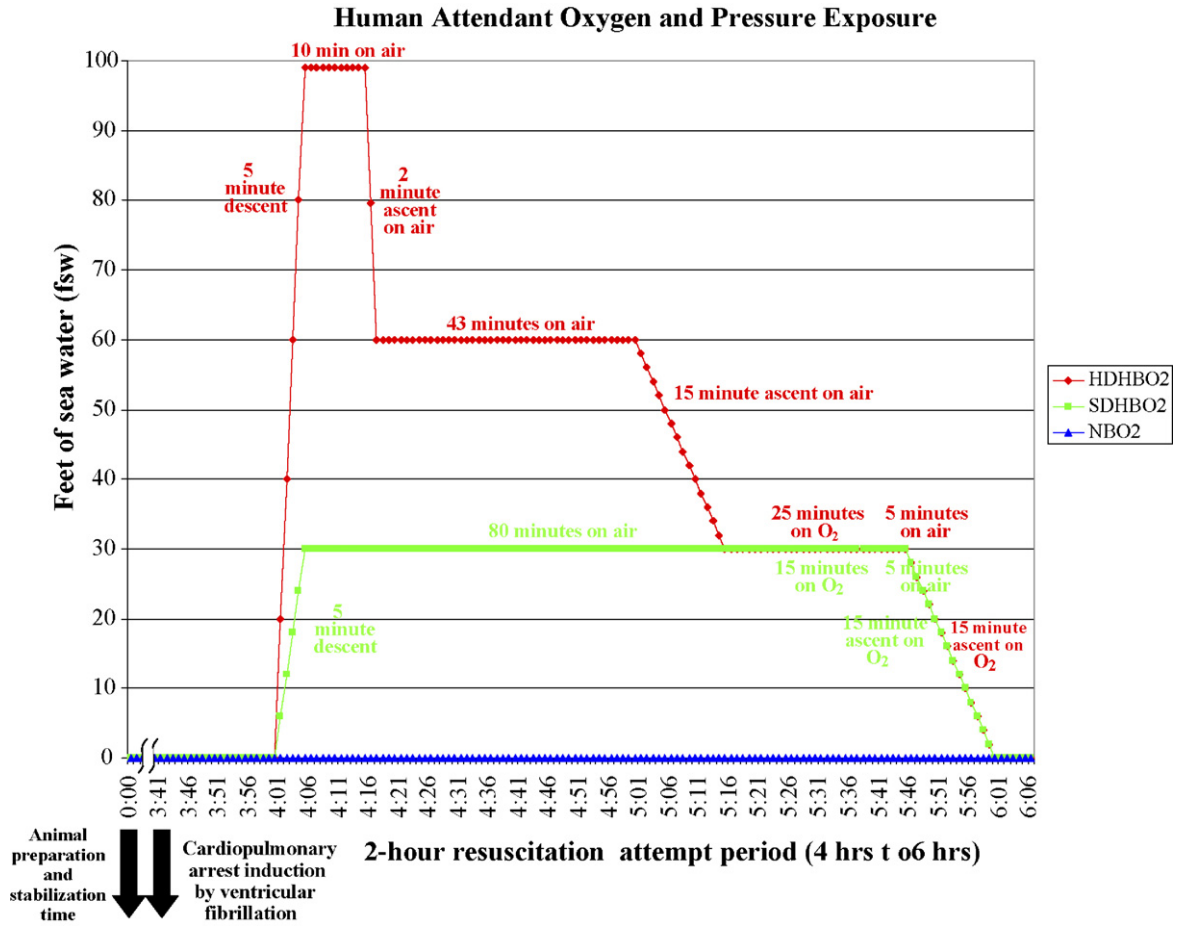

Figure 8 Human attendant oxygen and pressure exposure. In no instance did the human attendants or the treated animals in any of the groups exceed UPTD of greater than 300. UPTD of 300 or less are not associated with pulmonary oxygen toxicity damage.

disabling of beta-2 integrin adhesion of leukocytes to the ischemically damaged endothelium.[45] Compartment pressure of ischemic, edematous CNS tissue was also decreased by HBO_2.[46] HBO_2 can also ameliorate reperfusion dysrhythmias in reperfused, damaged myocardium.[37] Oxygen ventilation of critically ill or injured patients in the hyperbaric environment may assist resuscitation by providing oxygen in enough supply to help the reduction of an acutely accumulated oxygen debt of the cardiopulmonary arrest in a timely fashion.[47]

To illustrate, consider the following sequence of formulas to derive oxygen consumption (VO_2) and oxygen delivery (DO_2):

(1) $VO_2 = (CaO_2 - CvO_2) \times CI$ where CaO_2 = (oxygen content arterial blood) or [(mmHg $O_2 \times 0.003$) + (14 g/dL $\times$ O_2% sat)], CvO_2 = [oxygen content venous blood] or [(mmHg $O_2 \times 0.003$) + (14 g/dL $\times$ O_2% sat)], CI = Cardiac index (CO/body surface area (BSA) in m^2) or CO/m^2.

(2) $DO_2 = CI \times CaO_2$.

In this experiment only the swine in the $HDHBO_2$ group had the ability to maintain a sustained return of MAP $\geq$50 mmHg without the DMVAD engaged (again, all the animals in the NBO_2 or $SDHBO_2$ groups could not achieve this goal either with or without the DMVAD engaged). Swine at rest may consume 6.5 mL of oxygen per kg per minute[48,49]

Table 5 Accumulated pulmonary oxygen toxicity dose (UPTD) scores

	Total swine UPTD per resuscitation exposure to oxygen	Total human attendant UPTD per resuscitative attempt
NBO_2 group	120 UPTDs	N/A
$SDHBO_2$ group	228 UPTDs	93 UPTDs
$HDHBO_2$ group	300 UPTDs	242 UPTDs

In this experiment, neither the swine in any group nor the accompanying human attendants for any group had accumulated UPTD scores that exceeded 300. Pulmonary injury is not expected to be produced in patients with 300 or less UPTDs accumulated during any hyperbaric or normobaric oxygen exposure.

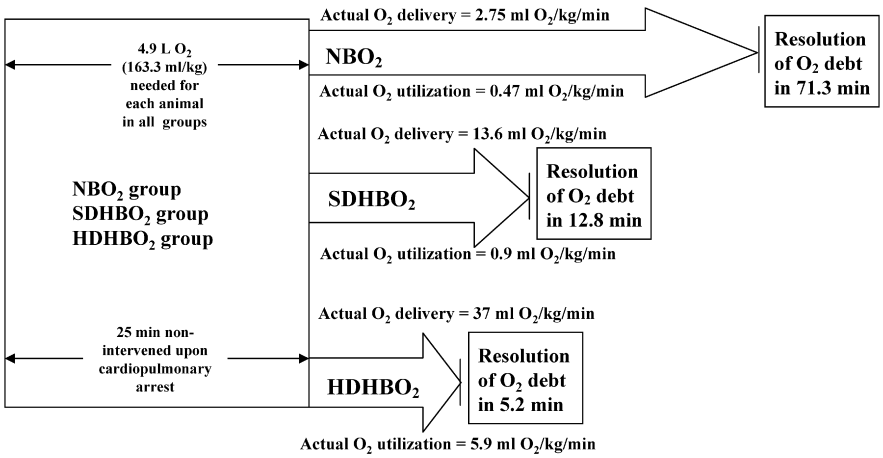

Figure 9 Estimate of resolution of oxygen debt by dose response of oxygen in ALS after a 25 min, non-intervened upon cardiopulmonary arrest. Basal oxygen consumption for swine is reported as being 6.5 mL O_2/(kg min).[50] The basal oxygen requirement was estimated by the assumed oxygen requirement of 6.5 mL O_2/(kg min) over 25 min of cardiopulmonary arrest followed by 120 min of resuscitative effort with a variable measured oxygen uptake for each group (see calculations for same in appendix): 30 kg × 6.5 mL/min = 195 mL O_2/min. Based on calculations taken on actual measurements made on the animals over the 120-min resuscitation, it would take 71 min into the resuscitation to satisfy the hypometabolic accumulating oxygen debt for the NBO_2 group animals, 13 min for the $SDHBO_2$ animals, and just 5 min for the more normally metabolic oxygen consumption rate of the $HDHBO_2$ animals.

or for these animals whose weights averaged 30 kg (±2 kg) a consumption which average 6.5 × 30 kg = 195 mmHg oxygen per minute. Thirty-five minutes after the resuscitative effort began (or at 1 h after the cardiopulmonary arrest was imposed), the swine in the $HDHBO_2$ group had COs which averaged 3.01 L/min (or CI of 5.79 L/min). The ideal calculated average DO_2 for this group of animals would have been $DO_2 = CI \times CaO_2$ or $[(14\,Hgb/dL \times 1.36\,mL) + (0.003\,mL \times 2953\,mmHg)] \times 10 \times 5.79\,L/min = 1633\,L\ O_2/min$. The animals in the NBO_2 and $SDHBO_2$ group had essentially no oxygen delivery without the assistance of the DMVAD because they had no CO (CI) on their own. If the DMVAD were engaged, reduction of the oxygen debts would never be reduced over the resuscitative 2-h period in the NBO_2 group or the $SDHBO_2$ group during the 2-h resuscitative attempt. In the $HDHBO_2$ group (without the need of the DMVAD in five of the six animals), the oxygen debt would be reduced in 5 min given the oxygen ventilation profile administered (Figure 9). See appendix for the entire calculation of VO_2, DO_2 and oxygen debt reduction for each animal group from real measured values.

Ideally the $HDHBO_2$ swine at 4.0 ATA of pressure while being ventilated with 100% oxygen would potentially have the approximate CaO_2 of 28.2 vol.%, while the $SDHBO_2$ group and NBO_2 group would have at 1.9 and 1.0 ATA respectively of 23.6 and 21.32 vol.% O_2 (see Table 6).

Measured hemoglobin saturations in arterial and venous blood were essentially normal in all animals of all groups pre-arrest. The measured hemoglobin saturations in the 2-h period of resuscitative effort reflected dose response to administered oxygen, but were diminished in all groups (Tables 7 and 8). Perhaps the oxygen carrying capacity

Table 6 Average of measured CaO_2 determinations by group for pre-arrest and 1 h, post-induction of ventricular fibrillation periods

	Average baseline CaO_2 pre-arrest (vol.%)	Average CaO_2 1 h post-induction of ventricular fibrillation
NBO_2 group	19.86	11.32
$SDHBO_2$ group	19.65	14.79
$HDHBO_2$ group	20.03	19.57

Table 7 Average of SvO_2 determinations by group for pre-arrest and 1 h, post-induction of ventricular fibrillation periods

	Average baseline SvO_2 pre-arrest (%)	Average baseline SvO_2 1 h post-induction of ventricular fibrillation (%)
NBO_2 group	66	46
$SDHBO_2$ group	58	65
$HDHBO_2$ group	81	74

Table 8 Average of SaO_2 determinations by group for pre-arrest and 1 h, post-induction of ventricular fibrillation periods

	Average baseline SaO_2 pre-arrest (%)	Average baseline SaO_2 1 h post-induction of ventricular fibrillation (%)
NBO_2 group	98	58
$SDHBO_2$ group	97	70
$HDHBO_2$ group	99	87

of the hemoglobin in prolonged normothermic cardiopulmonary arrest is severely impaired by acidosis as well as other unknown factors. The high level of dissolved oxygen in plasma in the $HDHBO_2$ group may bridge this problem in part.

Measured CaO_2 in this experiment was consistently less than calculated CaO_2 as would be expected clinically, but the point is made that the CaO_2 is high in the $HDHBO_2$ group by contribution of the dissolved oxygen in the plasma (almost 7 vol.% more than that achievable at 1.0 ATA/Abs).

In 1959, Boerema conducted a study to demonstrate the impact on oxygen delivery by HBO_2 ventilation suggested by similar calculations. He exsanguinated swine, then restored their intravascular volume with IV administered D5/Ringers' lactate/dextran solution. He administered HBO_2 at 3.0 ATA and normalized the animals' EKG's and vital signs even though the animal had hemoglobin levels as low as 0.4 g/dL. The plasma compartment contained enough dissolved oxygen to sustain vital function of organs. The animals, alert with normalized vital signs, were re-transfused with their shed blood, brought to surface after 15 min, and then returned to market.[51]

The present study shows that short-term, $HDHBO_2$ is an effective resuscitation tool and is safe in a small multiplace hyperbaric chamber. Perhaps, as in pediatric IV fluid resuscitation for dehydration, a similar use of resuscitation, restoration, and maintenance dosing of oxygen in treatment of oxygen debt will be more manageable through oxygen dosing in a hyperbaric environment. Furthermore, operationally in our clinical experience, it appears that $HDHBO_2$ complements and does not detract from standard closed- or open-chest ACLS. All of the procedural and monitoring activity used in ACLS done at 1.0 ATA can be readily be done in a small multiplace hyperbaric chamber in or out of a hospital.[52] A rehearsed team can easily load a patient in cardiopulmonary arrest into a small multiplace chamber in the pre-hospital or hospital setting without interrupting CPR or ACLS.

We intend that future experiments will explore long-term survival after $HDHBO_2$-assisted CPR/ACLS. It is important to determine if the use of a single $HDHBO_2$ treatment exposure during resuscitation is sufficient for survival or whether additional $SDHBO_2$ or low-dose HBO_2 (less than 1.9 ATA oxygen) serial daily treatments (''tailing $LDHBO_2$ treatments'') given after the resuscitative $HDHBO_2$ would generate a better recovery. In addition, further oxygen dose response studies are necessary to determine the effect of either hyperbaric air or oxygen since we have found evidence that both are more effective at 6.0 ATA than at 4.0 ATA oxygen (data not included). Concomitant use of hypothermia with HBO_2 should be researched. Lastly, the closed-chest model for $HDHBO_2$-assisted ACLS needs verification of efficacy because of greater ease of applying closed chest compressions in ACLS in more common clinical settings.

Conclusion

Ventilation of swine with $HDHBO_2$ when used as an element of ACLS in the circulatory phase of a prolonged, normothermic, non-intervened-upon cardiopulmonary arrest confers an improved rate of continuously sustainable ROSC as compared to that of ventilation with NBO_2 or $SDHBO_2$.

Conflicts of interest

Authors Keith Van Meter and Fred Kreidt have a patent pending on a hyperbaric medical resuscitation system.

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Appendix A. Calculations of VO_2, DO_2, and oxygen debt reduction for each animal group from measured experimental values

A.1. List of symbols

BSA	body surface area (m^2)
CaO_2	oxygen capacity of arterial blood (mL of oxygen/L of blood)
CI	cardiac index (cardiac output/min $\times$ surface area of animal) L/min $\times$ m^2
CO	cardiac output (L/min)
CvO_2	oxygen capacity of venous blood (mL of oxygen/L of blood)
DO_2	oxygen delivery (mL of oxygen/L of blood)
DMVAD	direct mechanical ventricular assist device
fsw	feet of seawater pressure

$HDHBO_2$ high-dose hyperbaric oxygen
Hgb hemoglobin
NBO_2 normobaric oxygen
SaO_2 oxygen hemoglobin saturation of the arterial blood (%)
$SDHBO_2$ standard-dose hyperbaric oxygen
SvO_2 oxygen hemoglobin saturation of the venous blood (%)
VO_2 oxygen utilization (mL of oxygen/L of blood)

A.2. Formulae

Formula 1:

$$\text{Oxygen delivery} = DO_2 = (CaO_2) \times CI$$

where CaO_2 = (oxygen carried by the hemoglobin in blood) + (oxygen saturated in the blood plasma), $CaO_2 = [\%SaO_2 \times$ (concentration of Hgb (g)/dL of blood) $\times$ (oxygen carrying capacity of Hbg/g of protein) $\times$ (conversion dL to L)] + [(0.003 cm^3/dL $\times$ partial pressure of oxygen) $\times$ (conversion dL to L)], $CaO_2 = [(\%SaO_2 \times 14\,g$ Hgb/dL $\times$ 1.36 mL oxygen/g Hgb) $\times$ (10dL/L)] + (0.003 cm^3/dL $\times$ partial pressure of oxygen mmHg) $\times$ (10 dL/L)]

Formula 2:

$$\text{Oxygen uptake} = VO_2 = (CaO_2 - CvO_2) \times CI$$

where $CaO_2 = [(\%SaO_2 \times 14\,g$ Hgb/dL $\times$ 1.36 mL oxygen/g Hgb) $\times$ (10 dL/L)] + [(0.003 cm^3/dL $\times$ partial pressure of oxygen mmHg) $\times$ (10 dL/L)], $CvO_2 = [(\%SvO_2 \times$ (concentration of Hgb (g)/dL of blood) $\times$ (oxygen carrying capacity of Hbg/g of protein)] + [(0.003 cm^3/dL $\times$ partial pressure of oxygen) $\times$ (10 dL/L)], $CvO_2 = [(\%SvO_2 \times 14\,g$ Hgb/dL $\times$ 1.36 mL oxygen/g Hgb) $\times$ (10 dL/L)] + [(0.003 cm^3/dL $\times$ partial pressure of oxygen mmHg) $\times$ (10 dL/L)].

Actual measurements #1 (average group arterial saturations of Hgb (SaO_2's) 1 h post-induction of ventricular fibrillation/35 min into resuscitation)

NBO_2 group	SaO_2 = 58%
$SDHBO_2$ group	SaO_2 = 70%
$HDHBO_2$ group	SaO_2 = 87% (at all times except SaO_2 = 100% @ 99 fsw/17 min)

A.3. Measured experimental values

Actual measurements #2 (Average group venous saturations of Hgb (SvO_2's) 1 h post-induction of ventricular fibrillation/35 min into resuscitation)

NBO_2 group	SvO_2 = 46%
$SDHBO_2$ group	SvO_2 = 66%
$HDHBO_2$ group	SvO_2 = 74%

Actual measurements #3 (average group cardiac output 1 h after induction of ventricular fibrillation/35 min into resuscitation)

	Group average CO	Group average CI (or CO/0.52 m^2)
NBO_2 group with DMVAD engaged	0.38 L/min	0.73 L/min
$SDHBO_2$ group with DMVAD engaged	1.44 L/min	2.77 L/min
$HDHBO_2$ group with DMVAD engaged (1 animal) with DMVAD not engaged (5 animals)	3.01 L/min	5.79 L/min

Actual measurements #4 (average arterial blood gas determinations for each group)

Note: 1 h into the period of resuscitative effort (1 h post-induction of ventricular fibrillation/35 min into resuscitation), both the NBO_2 and $SDHBO_2$ groups were at the uniform pressure of 1.0 and 1.9 ATA during the 2-h period (except for a very short period of pressurization and depressurization for the $SDHBO_2$ group considered negligible). The $HDHBO_2$ group required for safe decompression a multilevel dive allowing for distinctly different arterial gas saturations at each level (i.e. 99 fsw for 17 min, 60 fsw for 43 min, and 30 fsw for 60 min).

	99 fsw	60 fsw	30 fsw	Surface (0 fsw)
NBO_2 group	n/a	n/a	n/a	219 mmHg
$SDHBO_2$ group	n/a	n/a	504 mmHg	n/a
$HDHBO_2$ group	1338 mmHg	1000 mmHg	614 mmHg	n/a

A.4. Calculations of DO_2

Pressure time exposure $\times$ CI $\times$ CaO_2 or CI $\times$ [($SaO_2 \times$ Hgb 14 g/dL) $\times$ 10 + (ambient pressure mmHg $\times$ 0.003 mL)] = DO_2 over time.

Group NBO_2:

- 120 min $\times$ [(58% $SaO_2 \times$ 14 g/dL Hgb $\times$ 1.36 mL O_2) + (219 mmHg $\times$ 0.003)] $\times$ 10 $\times$ 0.73 L/min = DO_2 9.9 L over 120 min.

Group $SDHBO_2$:

- 105 min $\times$ [(70% $SaO_2 \times$ 14 g/dL Hgb $\times$ 1.36 mL O_2) + (504 mmHg $\times$ 0.003)] $\times$ 10 $\times$ 2.77 L/min = DO_2 43.16 L.
- 15 min $\times$ [(70% $SaO_2 \times$ 14 g/dL Hgb $\times$ 1.36 mL O_2) + (504 mmHg $\times$ 0.003)] $\times$ 10 $\times$ 2.77 L/min = DO_2 6.16 L.
- 43.16 L + 6.16 L = 49.32 L over 120 min.

Group $HDHBO_2$:

- 17 min $\times$ [(100% $SaO_2 \times$ 14 g/dL Hgb $\times$ 1.36 mL O_2) + (1338 mmHg $\times$ 0.003)] $\times$ 10 $\times$ 5.79 L/min = DO_2 22.69 L.
- 43 min $\times$ [(87% $SaO_2 \times$ 14 g/dL Hgb $\times$ 1.36 mL O_2) + (1000 mmHg $\times$ 0.003)] $\times$ 10 $\times$ 5.79 L/min = DO_2 48.7 L.
- 15 min $\times$ [(87% $SaO_2 \times$ 14 g/dL Hgb $\times$ 1.36 mL O_2) + (614 mmHg $\times$ 0.003)] $\times$ 10 $\times$ 5.79 L/min = DO_2 15.98 L.

- 30 min × [(87% SaO_2 × 14 g/dL Hgb × 1.36 mL O_2) + (614 mmHg × 0.003)] × 10 × 5.79 L/min = DO_2 31.96 L.
- 15 min × [(87% SaO_2 × 14 g/dL Hgb × 1.36 mL O_2) + (614 mmHg × 0.003)] × 10 × 5.79 L/min = DO_2 15.98 L.
- 22.69 L + 48.7 L + 15.98 L + 31.96 L + 15.98 L = 135.31 L over 120 min.

A.5. Calculations of VO_2

The swine by *a priori* assumption utilized 6.5 mL/(kg min) of oxygen at baseline pre-arrest[49] and in worst case *a priori* incurred an oxygen debt of 6.5 mL/(kg min) during the 25-min arrest. The swine may have incurred an oxygen consumption rate of 6.5 mL/(kg min) during the 2-h resuscitative attempt to follow, but our measurements of actual arterial oxygen tension and SvO_2 and SaO_2 indicate a much-reduced oxygen consumption during this time period.

Because oxygen delivery is not necessarily the same as oxygen drop-off or utilization, the matter may best be discussed further. The assumption is made in the post-arrest period that either a ROSC animal with a spontaneously beating heart producing a MAP on its own of $\geq$50 mmHg or in a PEA animal (MAP $\leq$ 50 mmHg) needing DMVAD assistance, that the reduced CO (or CI) would produce more of a mixed sate of arterial and venous oxygen gas tension. It is highly likely that post-arrest ischemically injured tissue would have less oxygen uptake.

Given these assumptions, the following set of equations for each animal group would more nearly calculate the actual oxygen delivery and utilization from measured data from this experiment:

A.5.1. Average CvO_2 calculations for each group over 2-h resuscitation period

Group NBO_2:

- (46% × 14 g/dL × 1.36 mL O_2/dL) + (219 mmHg O_2 × 0.003) = 9.42 vol.%.

Group $SDHBO_2$:

- (65% × 14 g/dL × 1.36 mL O_2/dL) + (504 mmHg O_2 × 0.003) = 13.89 vol.%.
- (65% × 14 g/dL × 1.36 mL O_2/dL) + (504 mmHg O_2 × 0.003) = 13.89 vol.%.

Group $HDHBO_2$:

- (74% × 14 g/dL × 1.36 mL O_2/dL) + (1338 mmHg O_2 × 0.003) = 18.10 vol.%.
- (74% × 14 g/dL × 1.36 mL O_2/dL) + (1000 mmHg O_2 × 0.003) = 17.09 vol.%.
- (74% × 14 g/dL × 1.36 mL O_2/dL) + (614 mmHg O_2 × 0.003) = 15.94 vol.%.
- (74% × 14 g/dL × 1.36 mL O_2/dL) + (614 mmHg O_2 × 0.003) = 15.94 vol.%.
- (74% × 14 g/dL × 1.36 mL O_2/dL) + (614 mmHg O_2 × 0.003) = 15.94 vol.%.

A.5.2. Average CaO_2 calculations for each group over the 2-h resuscitation period

Group NBO_2:

- (56% × 14 g/dL × 1.36 mL O_2/dL) + (219 mmHg O_2 × 0.003) = 11.32 vol.%.

Group $SDHBO_2$:

- (70% × 14 g/dL × 1.36 mL O_2/dL) + (504 mmHg O_2 × 0.003) = 14.84 vol.%.
- (70% × 14 g/dL × 1.36 mL O_2/dL) + (504 mmHg O_2 × 0.003) = 14.84 vol.%.

Group $HDHBO_2$:

- (100% × 14 g/dL × 1.36 mL O_2/dL) + (1338 mmHg O_2 × 0.003) = 23.07 vol.%.
- (87% × 14 g/dL × 1.36 mL O_2/dL) + (1000 mmHg O_2 × 0.003) = 19.56 vol.%.
- (87% × 14 g/dL × 1.36 mL O_2/dL) + (614 mmHg O_2 × 0.003) = 18.40 vol.%.
- (87% × 14 g/dL × 1.36 mL O_2/dL) + (614 mmHg O_2 × 0.003) = 18.40 vol.%.
- (87% × 14 g/dL × 1.36 mL O_2/dL) + (614 mmHg O_2 × 0.003) = 18.40 vol.%.

A.5.3. Calculation of total VO_2

The calculation used below is as follows

Time exposure to pressure × ($CaO_2 - CvO_2$) × CI = VO_2 over time of 120 min.

Group NBO_2:

- 120 min × 10 × (11.32—9.42 vol.%) × 0.73 L/min = 1.66 L over 120 min.

Group $SDHBO_2$:

- 105 min × 10 × (14.84—13.89 vol.%) × 2.77 L/min = 2.76 L.
- 15 min × 10 × (14.84—13.89 vol.%) × 2.77 L/min = 0.395 L.
- 2.76 L + 0.395 L = 3.2 L over 120 min.

Group $HDHBO_2$:

- 17 min × 10 × (23.07—18.10 vol.%) × 5.79 L/min = 4.89 L.
- 43 min × 10 × (19.56—17.09 vol.%) × 5.79 L/min = 6.15 L.
- 15 min × 10 × (18.40—15.49 vol.%) × 5.79 L/min = 2.53 L.
- 30 min × 10 × (18.40—15.49 vol.%) × 5.79 L/min = 5.05 L.
- 15 min × 10 × (18.40—15.49 vol.%) × 5.79 L/min = 2.53 L.
- 4.89 L + 6.15 L + 2.53 L + 5.05 L + 2.53 L = 21.15 L over 120 min.

DO_2 and VO_2 over 2-h resuscitative period:

	DO_2 (L)	VO_2 (L)
Group NBO_2 average	9.9	1.7
Group $SDHBO_2$ average	49.3	3.2
Group $HDHBO_2$ average	135.3	21.2

VO_2 = 6.5 mL/(kg min) × 30 kg × 25 min = 4.875 L.

VO_2 = 6.5 mL/(kg min) × 30 kg × 120 min = 23.4 L is probably way over-estimated and is more realistically as measured less (see anteceding table above).

DO_2 for NBO_2 group: 9.9 L/120 min = 0.083 L/min or 2.75 mL O_2/(kg min).
DO_2 for $SDHBO_2$ group: 49.3 L/120 min = 0.410 L/min or 13.6 mL O_2/(kg min).
DO_2 for $HDHBO_2$ group: 135.3 L/120 min = 1.128 L/min or 37.6 mL O_2/(kg min).

VO_2 for NBO_2 group: 1.7 L/120 min = 0.014 L/min or 0.47 mL O_2/(kg min).
VO_2 for $SDHBO_2$ group: 3.2 L/120 min = 0.027 L/min or 0.9 mL O_2/(kg min).
VO_2 for $HDHBO_2$ group: 21.2 L/120 min = 0.177 L/min or 5.9 mL O_2/(kg min) (see Figure 9).

A.6. Calculations for resolution of oxygen debt

Figure 9 assumes the following:

a. VO_2 during arrest is 6.5 ml of O_2/kg-min.
b. From the calculations, DO_2 results from each group are as follows:
   NBO_2: 2.75 ml of O_2/kg/min (9.9 L × 1000 ml/L)/(30 kg × 120 min)
   $SDHBO_2$: 13.6 ml of O_2/kg/min (49.3 L × 1000 ml/L)/(30 kg × 120 min)
   $HDHBO_2$: 37 ml of O_2/min/min (135.5 L × 1000 ml/L)/(30 kg × 120 min)
c. From the calculations (assuming utilization of oxygen is provided by hemoglobin only), VO_2 results during the 120-minute recovery period from each group are as follows:
   NBO_2: 0.47 ml of O_2/kg/min
   $SDHBO_2$: 0.9 ml of O_2/kg/min
   $HDHBO_2$: 5.9 ml of O_2/kg/min

The 25 min oxygen debt (due to the arrest) to be resolved is 4.9 L of oxygen for the entire animal or 163.3 ml/kg.

Let us consider Figure 9, group by group:

NBO_2:

DO_2 = 2.75 ml of O_2/kg/min
VO_2 = 0.47 ml of O_2/kg/min
Utilization is not greater than delivery; therefore, the 163.3 ml of oxygen debt will be resolved in 71 min (163.3/(2.75 − 0.47)).

$SDHBO_2$:

DO_2 = 13.6 ml of O_2/kg/min
VO_2 = 0.9 ml of O_2/gk/min
Utilization is not greater than delivery; therefore, the 163.3 ml of oxygen debt will be resolved in 12.9 min (163.3/(13.6 − 0.9)).

$HDHBO_2$:

DO_2 = 37 ml of O_2/kg/min
VO_2 = 5.9 ml of O_2/kg/min
Utilization is not greater than delivery; therefore the 163.3 ml of oxygen debt will be resolved in 5.25 min (163.3/(37 − 5.9)).

References

1. Safar P, Escarraga LA, Chang F. Upper airway obstruction in the unconscious patient. J Appl Physiol 1959;14:760—4.
2. Safar P, Escarraga LA, Elam JO. A comparison of the mouth-to-mouth and mouth-to-airway methods of artificial respiration with the chest-pressure arm-lift methods. N Engl J Med 1958;258:671—7.
3. Kouwenhoven WB, Jude JR, Knickerbocker GG. Closed-chest cardiac massage. JAMA 1960;173:1064—7.
4. Zoll PM, Linenthal AJ, Gibson W, Paul MH, Norman LR. Termination of ventricular fibrillation in man by externally applied electric countershock. N Engl J Med 1956;254:727—32.
5. Pearson JW, Redding JS. Epinephrine in cardiac resuscitation. Am Heart J 1963;66:210—4.
6. Moon AJ, Williams KG, Hopkinson WI. A patient with coronary thrombosis treated with hyperbaric oxygen. Lancet 1964;1:18—20.
7. Gordon AS. Standards for cardiopulmonary resuscitation (CPR) and emergency cardiac care (ECC). JAMA 1974;227:836—66.
8. Eisenberg MS, Bergner L, Hallstrom A. Cardiac resuscitation in the community: importance of rapid provision and implications for program planning. JAMA 1979;241:1905—7.
9. Thel MC, O'Connor CM. Cardiopulmonary resuscitation: historical perspective to recent investigations. Am Heart J 1999;137:39—48.
10. Stiell IG, Wells GA, Field B, et al. Advanced cardiac life support in out-of-hospital cardiac arrest. N Engl J Med 2004;351:647—56.
11. Eckstein M, Stratton SJ, Chan LS. Cardiac arrest resuscitation evaluation in Los Angeles: CARE-LA. Ann Emerg Med 2005;45:504—9.
12. Van Meter K, Weiss L, Harch PG. HBO in emergency medicine. In: Jain KK, editor. Textbook of Hyperbaric Medicine. 4th ed. Cambridge, MA: Hogrefe & Huber; 2004. p. 421—49.
13. Hossmann KA, Sato K. Recovery of neuronal function after prolonged cerebral ischemia. Science 1970;168:375—6.
14. Hossmann KA, Kleihues P. Reversibility of ischemic brain damage. Arch Neurol 1973;29:375—84.
15. Hossmann KA, Lechtape-Grüter H, Hossman V. The role of cerebral blood flow for the recovery of the brain after prolonged ischemia. Z Neurol 1973;204:281—99.
16. Ames A, Nesbett FB. Pathophysiology of ischemic cell death, part I—III. Stroke 1983;14:219—40.
17. Hossmann KA, Schmidt-Kastner R, Grosse Ophoff B. Recovery of integrative central nervous function after one hour global cerebro-circulatory arrest in normothermic cat. J Neurol Sci 1987;77:305—20.
18. Safar P. Resuscitation from clinical death: pathophysiologic limits and therapeutic potentials. Crit Care Med 1988;16:923—41.
19. Jennings RB, Reimer KA, Steenbergen C. Complete global myocardial ischemia in dogs. Crit Care Med 1988;16:988—96.
20. Radovsky A, Safar P, Sterz F, Leonov Y. Morphology of myocardial necrosis after 15 or 17 minutes of ventricular fibrillation cardiac arrest and cardiopulmonary bypass in dogs. Ann Emerg Med 1989;18:454.
21. Reich H, Angelos M, Safar P, Sterz F, Leonov Y. Cardiac resuscitability with cardiopulmonary bypass after increas-

ing ventricular fibrillation times in dogs. Ann Emerg Med 1990;19:889—90.
22. Gazmuri RJ, Weil MH, Terwilliger K, Shah DM, Duggal C, Tang W. Extracorporeal circulation as an alternative to open-chest cardiac compression for cardiac resuscitation. Chest 1992;102(6):1846—52.
23. Maldonado FA, Weil MH, Tang W, et al. Myocardial hypercarbic acidosis reduces cardiac resuscibility. Anesthes 1993;78(2):343—52.
24. Wenzel V, Lindner KH, Prengel AW, et al. Vasopressin improves vital organ blood flow after prolonged cardiac arrest with post-countershock pulseless electrical activity in pigs. Crit Care Med 1999;27(3):486—92.
25. Krismer AC, Lindner KH, Kornberger R, et al. Cardiopulmonary resuscitation during severe hypothermia in pigs: does epinephrine or vasopressin increase coronary perfusion pressure? Anesthes Analg 2000;90(1):69—73.
26. Vayrynen T, Kuisma M, Maatta T, Boyd J. Medical futility in asystolic out-of-hospital cardiac arrest. Acta Anaesthsiol Scand 2008;52:81—7.
27. Anstadt MP, Malone JP, Brown GR, Nolan DS, Quinones JD, Anstadt GL. Direct mechanical ventricular assistance promotes salvage of ischemic myocardium. Trans Am Soc Artif Intern Organs 1987;33:720—5.
28. Brown CG, Schlaifer J, Jenkins J, et al. Effect of direct mechanical ventricular assistance on myocardial hemodynamics during ventricular fibrillation. Crit Care Med 1989;17(11):1175—80.
29. Bjørnstad H, Mortensen E, Sager G, Refsum H. Effect of bretylium tosylate on ventricular fibrillation threshold during hypothermia in dogs. Am J Emerg Med 1994;12:407—12.
30. Mitchell SJ. Lidocaine for the treatment of decompression illness: a review of the literature. Undersea Hyperbar Med 2001;28:165.
31. Wasowicz W, Neve J, Peretz A. Optimized steps in fluorometric determination of thiobarbituric acid-reactive substances in serum: importance of extraction pH and influence of sample preservation and storage. Clin Chem 1993;39:2522—6.
32. Chatizipanteli K, Alonso DF, Kraydieh S, Dietrich WD. Importance of post-traumatic hypothermia and hyperthermia on the inflammatory response after fluid percussion brain injury: biochemical and immunocytochemical studies. J Cerebral Blood Flow Metab 2000;20:531—42.
33. Bella BD, Valentini MA, Comporti M, Maellaro E. Cisplatin-induced apoptosis in melanoma cells: role of caspase-3 and caspase-7 in APAF-1 proteolytic cleavage and in execution of the degradative phases. Ann NY Acad Sci 2003;1010:200—4.
34. McCully JD, Wakiyama H, Cowan DB, Federman M, Parker RA, Levitsky S. Diazoxide amelioration of myocardial injury and mitochondrial damage during cardiac surgery. Ann Thorac Surg 2002;74:2138—46.
35. Milliken GA, Johnson DE. Analysis of messy data. Volume I: Designed experiments. Van Nostrand Reinhold Company: New York, p. 413—23.
36. Edward D, Berry JJ. Post-hoc comparisons by a method of simulation. Biometrics 1987;43:913—28.
37. Thomas MP, Brown LA, Sponseller DR, Williamson SE, Diaz JA, Guyton DP. Myocardial infarct size reduction by synergistic effect of hyperbaric oxygen and recombinant tissue plasminogen activator. Am Heart J 1990;120:791—800.
38. Hutchison JH, Kerr MM, Williams KG, Hopkinson WI. Hyperbaric oxygen in the resuscitation of the newborn. Lancet 1963;13:1019—22.
39. Smith G, Lawson DD. The protective effect of inhalation of oxygen at two atmospheres absolute pressure in acute coronary arterial occlusion. Surg Gynecol Obstet 1962;114:320—2.
40. Rosenthal RE, Silbergleit R, Hof PR, Haywood Y, Fiskum G. Hyperbaric oxygen reduces neuronal death and improves neurological outcome after canine cardiac arrest. Stroke 2003;34:1311—6.
41. Van Meter K, Gottlieb SF, Whidden SJ. Hyperbaric oxygen as an adjunct in ACLS in guinea pigs after 15 minutes of cardiopulmonary arrest (abstract). Undersea Biomed Res 1988;15(Suppl):55.
42. Hendricks PL, Hall DA, Hunter WL, Haley PJ. Extension of pulmonary oxygen tolerance in man at 2 ATA by intermittent oxygen exposure. J Appl Physiol 1977;42:593—9.
43. Lambertson CJ. Effects of hyperopia on organs and their tissues. In: Robin ED, editor. Extrapulmonary manifestations of respiratory disease: Lung biology in health and disease, vol. 8. New York: Marcel Deckker; 1979. p. 239—303.
44. Zamboni WA, Roth AC, Russel RC, Graham B, Suchy H, Kucan JO. Morphologic analysis of the microcirculation during reperfusion of ischemic skeletal muscle and the effect of hyperbaric oxygen. Plast Reconstr Surg 1993;91:1110—23.
45. Thom SR, Mendiguren I, Hardy K, et al. Inhibition of human neutrophil beta2-integrin-dependent adherence by hyperbaric O_2. Am J Physiol 1997;272:C770—7.
46. Miller JD, Ledingham IM, Jennett WB. Effects of hyperbaric oxygen on intracranial pressure and cerebral blood flow in experimental cerebral oedema. J Neurol Neurosurg Psychiatr 1970;33:745—55.
47. Van Meter KW. A systematic review of the application of hyperbaric oxygen in the treatment of severe anemia: an evidence-based approach. Undersea Hyperbar Med 2005;32:61—83.
48. Pointer RK, Brand MD. Cellular oxygen consumption depends on body mass. Am J Physiol 1995;269 (Regulatory Integrative Comp Physiol, 38:R226—R228).
49. Ozier Y, Grosse H, Baudin F, et al. Oxygen consumption by indirect colorimetry and Fick equation in swine: role of cardiac output. Ann Fr Anesth Reanim 1997;16(2):131—7.
50. Shoemaker WC, Appel PL, Kram HB. Tissue oxygen debt as a determinant of lethal and non-lethal post-operative organ failure. Crit Care Med 1989;16(11):1117—20.
51. Boerema PI, Meyne NG, Brummelkamp WK, et al. Life without blood: a study of the influence of high atmosphere pressure and hypothermia on dilution of the blood. J Cardiovas Surg 1960;1:133—46.
52. Van Meter K. Medical field management of the injured diver. Respiratory Care Clinics of North American. Hyperbar Med 1999;5(1):137—77.